

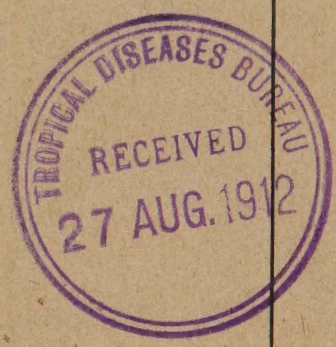
3286

Studies of Malaria in Panama: III. The Etiology of the Erythrolytic Hemoglobinuric Type of Blackwater Fever

A Preliminary Report

WALTER V. BREM, M.D.
LOS ANGELES

WELLCOME INSTITUTE LIBRARY	
Coll.	weTROmec
Call	Pam
No.	WC 750
	1912.
	B83s2



Reprinted from the Archives of Internal Medicine
Feb., 1912, Vol. 9, pp. 129-136

CHICAGO
AMERICAN MEDICAL ASSOCIATION
FIVE HUNDRED AND THIRTY-FIVE DEARBORN AVENUE
1912



22200128819

STUDIES OF MALARIA IN PANAMA: III. THE ETIOLOGY OF THE ERYTHROLYTIC HEMOGLOBINURIC TYPE OF BLACKWATER FEVER *

A PRELIMINARY REPORT

WALTER V. BREM, M.D.

LOS ANGELES

Last year, in a paper¹ read before this society, I proposed that the terms "blackwater fever," etc., be abandoned, and that the fever more or less closely connected with malaria and accompanied by hemoglobinuria should be divided into two groups, "pernicious malaria with hemoglobinuria" and "erythrolytic hemoglobinuria," the latter being the type most commonly meant by the old terms. I reported eight successive cases of pernicious malaria in which hemoglobinuria was demonstrated by the guaiac and turpentine test. The degree of hemoglobinuria varied from a mere trace, demonstrable only by the special test, to the intensity of black-water in two cases.

It was my idea then that the mechanism of the production of hemoglobinuria in the two types differed. I thought that in the first type the preceding hemoglobinemia was brought about by the destruction of innumerable erythrocytes, each by its infecting parasite, the unused hemoglobin being set free in the plasma. In the second type, the theory is generally accepted that there is an erythrolysis due to an unknown hemolysin.

Since the report was made, I have studied nineteen additional cases of pernicious malaria with reference to hemoglobinuria, making twenty-seven cases in all. In twenty-two of these hemoglobinuria was demonstrable. In eight instances there were traces only of hemoglobin in the urine; in seven the gross appearance of the urine suggested the probability of hemoglobin; in the other seven cases the hemoglobinuria was of black-water intensity. Four of the five patients without hemoglobinuria were comatose with but few parasites in the peripheral blood; two died and at autopsy the brain capillaries were found to be thrombosed and contained

*Read at the eighth annual meeting of the American Society of Tropical Medicine, New Orleans, May 18, 1911, being the result of studies in the Colon Hospital, Cristobal, C. Z.

1. Brem, W. V.: THE ARCHIVES INT. MED., 1911, vii, 153.

numerous parasites, while there were but few in the spleen and bone-marrow. The fifth patient had a heavy peripheral infection, about 9 per cent. of the corpuscles containing parasites. He was very ill, but did not become comatose and no hemoglobin could be demonstrated in his urine.

TABLE SHOWING RELATION BETWEEN THE NUMBER OF PARASITES IN THE PERIPHERAL BLOOD AND THE DEGREE OF HEMOGLOBINURIA

Infected Corpuscles Counted			Infected Corpuscles Estimated		
	Per Cent. Infected	Degree of Hemoglobinuria		Per Cent. Infected	Degree of Hemoglobinuria
1.	43.	++	15.	8-10	0
2.	29.	+	16.	5-10	++
3.	23.5	++	17.	5-10	+
4.	17.6	++	18.	5-10	+++
5.	15.6	+++	19.	5-10	+
6.	15.4	+++	20.	5-10	+
7.	12.6	+++	21.	5-10	++
8.	11.4	++	22.	2- 3	+
9.	9.	+++	23.	1- 2.5	0
10.	6.2	+	24.	1- 2.5	+
11.	3.2	+++	25.	1- 2.5	0
12.	2.3	0	26.	1- 2.5	+++
13.	2.	0	27.	1- 2.5	+
14.	15-20	+++			

+ = Trace; ++ = gross appearance of urine suggestive of hemoglobinuria; +++ = blackwater.

After the discovery that hemoglobinuria was an almost constant phenomenon in pernicious malarial infections, the question at once arose as to whether or not the fact could throw light on the etiology of erythrolytic hemoglobinuria. There were two of the cases which were difficult to classify. The coma with a moderate malarial infection (2 or 3 per cent. of infected corpuscles), and the absence of marked jaundice and vomiting seemed to place them in the pernicious group, while the intensity of the hemoglobinuria (blackwater) suggested the erythrolytic group. They were border-line cases and suggested strongly a connection between the two. Finally, a case that seemed to be even more of a clinical link occurred. The patient was admitted one afternoon, at which time it was found that 9 per cent. of his corpuscles were infected with young forms of estivo-autumnal parasites. The urine was not hemoglobinous. It was estimated that the group of parasites would sporulate during the evening of the following day, and it was thought that then hemoglobin might be demonstrated in the urine. The expected paroxysm of fever occurred late in the afternoon; it was accompanied by vomiting, and jaundice rapidly developed; at 9:30 p. m. the first urine was passed and was found to be typical blackwater; the parasites, which had been numerous all day, promptly disappeared and none could be found the following morning. The peripheral infection in this case was sufficient to classify it as pernicious, while the paroxysm, which came approximately at the

time calculated from the blood examination, was typical in every respect of erythrolytic hemoglobinuria, and was undoubtedly associated with sporulation of the large group of parasites.

Before this time we had had all grades of malarial infections, from mild up to 5 or 6 per cent. infections of the erythrocytes, associated with typical erythrolytic hemoglobinuria. In many of the most severe cases the parasites were few or absent, while in some of the mildest cases they were numerous. In order to see if the same lack of relation between the number of parasites in the peripheral blood and the degree of hemoglobinuria obtained in pernicious malaria with hemoglobinuria, the percentage of infected corpuscles in the pernicious cases was determined and compared with the degree of hemoglobinuria.

My original idea was that the degree of hemoglobinuria in pernicious infections would vary with the absolute number of parasites, but, as far as can be judged by the peripheral infection, the accompanying table shows that such is not the case. The absolute number of parasites may be considered as being equal to the sum of the parasites in the peripheral circulation and the internal organs. We know that a scarcity of parasites in the peripheral circulation does not indicate a scarcity in the internal organs, but a heavy peripheral infection does indicate a heavy internal infection. Granted that in all the light peripheral infections in the above table there was a heavy internal infection, it seems reasonable to assume that there was a still heavier internal infection associated with the heavy peripheral infections. Autopsy findings, also, indicate that this is the case. Therefore, for purposes of comparison, the peripheral infection may be considered a rough indicator of the absolute number of parasites. If the criterion of infection is valid, it is evident that the hemoglobinuria of pernicious malaria has this in common with that of erythrolytic hemoglobinuria, that the intensity of the hemoglobinuria is not directly related to the absolute number of malarial parasites. Further, it seems probable that in some pernicious infections the destruction of corpuscles and the liberation of hemoglobin is not confined to the infected erythrocytes.

These facts support the theory that malarial parasites produce an hemolysin that varies in the intensity of its action in different hosts or under different conditions of environment, or that some strains of malarial parasite may elaborate a larger quantity of or an especially powerful, hemolysin. That the malarial parasite does generate an hemolysin may be reasonably inferred from the fact that it utilizes the hemoglobin of the infected cell, probably as a food. Brown² has shown recently that malarial pigment is hematin, and this proves that the parasites or their products have a power to break up hemoglobin. He suggests that they possess a proteolytic enzyme. Whatever may be the nature of the sub-

2. Brown, W. H.: Jour. Exper. Med., 1911, xiii, No. 2, p. 290.

stance it probably acts, first, by dissolving the hemoglobin, which passes into the parasite by osmosis, and is there decomposed into globulin and hematin.

I have begun some experiments that so far seem to indicate that malarial organisms do generate an hemolysin. In several experiments malarial infected blood was quickly defibrinated while kept at about 37 C., and measured quantities were placed in sterile tubes as follows:

Tube 1. Defibrinated blood 0.8 c.c.

Tube 2. Defibrinated blood 0.8 c.c. plus 0.2 c.c. of 0.05 per cent. quinin in 0.9 per cent. salt solution.

Tube 3. Defibrinated blood 0.8 c.c. plus 0.2 c.c. of 0.1 per cent. quinin in 0.9 per cent. salt solution.

Tube 4. Defibrinated blood 0.8 c.c. plus 0.2 c.c. of 0.025 per cent. quinin in 0.9 per cent. salt solution.

The tubes were incubated for forty-eight hours, during which time they were shaken four times. Cultures on agar were then made to exclude contaminating hemophilic organisms. The tubes were centrifugalized and readings made, comparing hemolysis with that of controls of normal blood similarly treated. The quinin added to Tubes 2, 3, and 4 gave solutions of quinin 1 to 5,000, 1 to 10,000, and 1 to 20,000, respectively, approximating concentrations in the circulating blood when given therapeutically. The results, so far, may be summarized as follows:

1. Malarial defibrinated blood showed greater hemolysis than the same blood to which quinin had been added, and greater than the controls.

2. The quinin tubes showed degrees of hemolysis that varied in proportion to the strength of solution in one experiment, and in another in inverse proportion. In the latter instance, the parasites were not as promptly killed by the weaker solutions as they were in the former, and it seemed that parasitic hemolysis was added to quinin hemolysis, thus causing hemolysis to vary inversely as the strength of the quinin solution. None of the quinin tubes, however, showed as much hemolysis as the tubes with only defibrinated malarial blood.

3. Smears made from the defibrinated blood after incubation showed malarial parasites in various stages of degeneration. Many of the original unpigmented forms grew larger and became pigmented before death. These larger parasites were invariably found in corpuscles partly or completely hemolyzed. The majority of the parasites, however, appeared to have died quickly. In the stained specimen they appeared as round blue dots, the chromatin having lost its acidophilic power, and their corpuscles were never hemolyzed. In the strongest quinin solutions the parasites seemed to have been killed quickly and no hemolyzed corpuscles containing parasites could be found. Crescents retained a fairly normal appearance for forty-eight hours in defibrinated blood, but they were distorted into

bizarre shapes and spherical masses by the quinin, and their staining properties were markedly altered.

It may be said that the experiments demonstrate that malarial parasites or their products possess a certain power to hemolyze red corpuscles. Only the parasites that underwent some growth in the incubator exhibited this power. The experiments are too few to permit further conclusions, but they seem to indicate that quinin in salt solution, added to malarial defibrinated blood in quantities approximating therapeutic strengths, inhibits parasitic growth and parasitic hemolysis, and that the quinin itself produces some hemolysis in forty-eight hours, the hemolysis being of about the same intensity as that in control tubes of non-malarial blood.

Similar experiments have been carried out in seven cases of erythrolytic hemoglobinuria. The results showed no marked deviation from the normal. Corpuscles from different patients seem to vary slightly in their resistance to the quinin solutions.

In the seven cases of erythrolytic hemoglobinuria, the serums and corpuscles were tested against normal serums and corpuscles, and with serums and corpuscles of other hemoglobinuric patients. In no case has any hemolysin been demonstrated, the results agreeing with those of Barratt and Yorke.³ The hemoglobinuric bloods tested against normal bloods have fallen naturally in the agglutination groups determined by Moss.⁴

Group I—2 cases.
Group II—1 case.

Group III—0 cases.
Group IV—4 cases.

In certain instances I have found that hemoglobinuric serum possessed atypical agglutinating powers against corpuscles of other hemoglobinuric patients. For instance, the serum of Group I normally should not agglutinate corpuscles of any group, and the corpuscles of Group IV should not be agglutinated by any serum. But I found that the serum of both of the hemoglobinuric Group I patients agglutinated hemoglobinuric Group IV corpuscles. I do not yet know what significance this may have, but it is interesting to note in connection with it that in two instances I observed in smears from hemoglobinuric patients phagocytosis of red corpuscles. Ottenberg observed this latter phenomenon in smears from the blood of patients transfused with blood of donors belonging to another group, the corpuscles of which were agglutinated by the patient's serum. In the hemoglobinuric serum the atypical agglutination of other hemoglobinuric corpuscles and the autophagocytosis of erythrocytes suggest the presence of an agglutinin not present in normal blood. Hemolysins

3. Barratt, J. O. W., and Yorke, W.: *Ann. Trop. Med. and Parasit.*, 1909, iii, No. 1, p. 1-256.

4. Moss, M. L.: *Bull. Johns Hopkins Hosp.*, 1910, xxi, 63.

in normal serums have always been found accompanied by agglutinins, but the hemolysins can be destroyed (by destroying the complement) without affecting the agglutinin. It seemed possible, therefore, that in hemoglobinuria, the complement might have been absorbed and so the demonstration of an hemolysin prevented. But when complement was added to hemoglobinuric serums by adding serum of a normal person belonging to the same group, no hemolysis occurred. The hypothetical hemolysin appeared to have been completely absorbed, or used up during the process of erythrolysis.

Three guinea-pigs were inoculated with blood from three patients with erythrolytic hemoglobinuria. Two pigs that received 6 and 5 c.c. of blood died within thirty-six hours. The third received 5 c.c. and appeared ill for about two days, but recovered. None voided hemoglobinous urine and the autopsy urine did not yield a positive guaiac and turpentine reaction. At autopsy a somewhat greater quantity of bloody fluid was present in the peritoneum than had been injected, but there seemed to be no other lesions. A small quantity of heart's blood from each of the two dead pigs was injected into other pigs intraperitoneally. They remained unaffected.

Finally, one other experiment was made which I hesitate to report because it is open to certain objections, and because it has not been repeated on account of the fact that we have not since had a suitable case. As it is, however, the experiment is of such interest that it seems justifiable to present it.

The blood of a patient with a very heavy estivo-autumnal malarial infection was laked immediately with sterile distilled water, and the stromata of the corpuscles quickly thrown down with the centrifuge. A smear showed that many parasites were thrown down with the stromata. The supernatant fluid was removed and the sediment was ground with sterile sand and then was taken up with a small quantity of distilled water, with which it was allowed to remain in contact for some time. Then an equal quantity of 1.8 per cent. salt solution was added. It was thought that an extract of parasites in 0.9 per cent. salt solution might be obtained in this way. A small quantity of this extract was added to an equal quantity of a 5 per cent. suspension of normal corpuscles, and the mixture incubated over night. Complete hemolysis took place. A platinum loopful of the parasite extract was then added to 0.25 c.c. of a 5 per cent. suspension of normal corpuscles, and again hemolysis was complete after incubation over night. A control made with a similar extract of leukocytes showed no hemolysis. However, as a transplant from the parasite extract on agar showed the presence of a contaminating bacillus, which had probably produced the hemolysis, I lost interest in the experiment, and put aside the extract in the ice-chest. Several days later the extract was tested again against normal corpuscles and no hemolysis was obtained. The contaminating organism was still present. If

it had caused the hemolysis in the first tests, hemolysis should have occurred, also, in the last. As the first culture of the contaminating organism had been saved, it was inoculated into a 5 per cent. suspension of normal corpuscles. No hemolysis took place. This appears to exclude the possibility that the hemolysis in the first tests was due to the contaminating organism, and since no hemolysis was obtained with leukocytic extract, it seems plausible that the first hemolysis was due to an hemolysin derived from the estivo-autumnal malarial parasites.

If this experiment can be repeated, and if an hemolysin in solution can be obtained in considerable quantity from malarial organisms, an interesting field of experiment will be opened, and the question of the relation of malaria to erythrolytic hemoglobinuria should be definitely settled. It is probable that such an extract of parasites would in large quantities reproduce the phenomena of the disease in animals, and that in animals an antihemolysin might be stimulated by repeated small doses such as the infected man may receive during the incubation period. Quinin may inhibit the development of the antihemolysin. Numerous other experiments suggest themselves, a discussion of which at length would be premature.

SUMMARY

1. A consideration of pernicious malaria with hemoglobinuria, and the transition cases between it and erythrolytic hemoglobinuria, strongly inclines one to believe that both forms are due directly to an hemolysin produced by the malarial parasite. The estivo-autumnal organism is nearly always the one concerned.

2. The mechanisms of the production of the two types are, it is thought, essentially the same, but differ markedly, as a rule, in degree.

3. The small amount of experimental evidence so far collected tends to confirm the above views.

4. One of two explanations may account for the irregularity and infrequency of hemoglobinuria in malarial infections:

- A. Different strains of parasites may generate hemolysin that varies in quantity or intensity with the strain, or with environment that is inimical to the parasites, such as quinin or relative immunity to malaria resulting from previous infections.

- B. An antihemolysin may be formed, as a rule, during the incubation period of malarial infections, when gradually increasing doses of parasitic hemolysin are presumably being liberated. In this case quinin, exhaustion, exposure, etc., may inhibit the production of antihemolysin, especially in debilitated persons who have suffered from previous malaria. In pernicious infections, antihemolysin formation may be good, but the production of hemolysin by enormous numbers of parasites may be sufficient to more than neutralize it.

I wish to thank Col. W. C. Gorgas, Chief Sanitary Officer, for permission to publish this paper.

NOTE.—Since the above paper was written, Zeiler and I have obtained two more extracts of parasites from the different pernicious malarial infections. The

extracts were made like the one reported above, except that grinding of the sediment with sand was omitted. Both of these extracts contained a fairly strong hemolysin. Three parts of the extracts hemolyzed completely one part of a 5 per cent. suspension of erythrocytes in from twenty minutes to one hour. The hemolysin was thermolabile, its action was inhibited by one part of serum from normal persons, from one patient with pernicious malaria (the patient from whom one extract was obtained), and from two patients with erythrolytic hemoglobinuric fever. The antihemolysin in these serums was thermostabile. The serums were treated with quinin and tested for antihemolytic properties. The normal serums were unimpaired, but the serums from erythrolytic hemoglobinuric patients seemed to be weakened by the same quantity of quinin and in one instance hemolysis was complete in one hour. The experiments need confirmation and we hope to be able to report more exact quantitative work. They seem to reconcile the malarial and quinin views of erythrolytic hemoglobinuria and to connect it definitely with malaria. The therapeutic measure of transfusion with normal blood is obviously suggested.

423 Security Building.



